Format for ANSWERING REVIEWERS

September 25, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12825-Song review.doc).

Title: Mitochondria as therapeutic targets for cancer stem cells

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) ***Reviewer 1 (00225340) recommended to reinforce some concepts***.

As recommended, to reinforce the importance of mitochondria in CSCs field, we added the current situation of studies dealing with CSCs mitochondria in several cancer types which is summarized in table 2. Table 2 shows the need for more in-depth studies about mitochondrial features in various cancer types. Hereafter, CSCs studies must be focused on demonstrating the cause of the varying mitochondrial features according to cancer type. Thus, this review emphasized the importance and the necessity of mitochondria study in cancer stem cells.

We represented the current situation of CSCs studies on line 23 of page10 to line 11 of page 11, as follows:

Leukemia CSCs showed a low ROS level and reduced OXPHOS compared with that of non-CSCs [64]. However, Patro et al. reported that CSCs exhibited over-expressed

genes related to glucose uptake, oxidative phosphorylation, and fatty acid β-oxidation, indicating higher ability to direct pyruvate towards the TCA cycle [65]. As reported, ovarian CSCs showed higher mitochondrial ROS production and ΔΨm than non-CSCs. In addition, targeting mitochondrial biogenetics induced caspase-indepenent cell death in ovarian CSCs [66]. In glioma CSCs, a higher mitochondrial reserve capacity was measured as compared to the differentiated cells [67]. Glioblastoma CSCs also depend on OXPHOS for their energy production and survival [68]. Besides, breast CSCs have higher ATP content compared to their differentiated progeny [69]. Based on these studies, CSCs mitochondria showed the different roles and features according to the cancer type. A summary of the mitochondrial features between CSCs and non-CSCs according to cancer origin is highlighted in table 2. Although the mitochondrial features of CSCs in several cancers are not identical, CSCs mitochondria obviously differ from those of non-CSCs. Moreover, mitochondrial features of CSCs have not been clearly defined in other cancer types. Most importantly, little has been known about the mitochondrial features related to energy metabolism and the ROS/antioxidant enzyme system of CSCs in colon, stomach, liver, bone, and prostate cancer.

(2) ***Reviewer 2 (00396997) recommended to emphasize more about what is the difference of cancer stem cells mitochondria and non-cancer mitochondria.***

As recommended, we represented the difference of cancer stem cells mitochondria and non-cancer stem cells mitochondria in table 2.

what is the advantage to use mito-target drug to kill CSCs instead of using general anti-cancer drug?

Mitochondria are known to play a key role in apoptosis and can trigger cell death via several mechanisms, including the disruption of electron transport and energy metabolism, the release or activation of proteins that mediate apoptosis, and the alteration of the cellular redox potential. Therefore, by studying the mitochondrial features between CSCs and non-CSCs, we can identify targets to develop the effective anticancer drug for the elimination of CSCs. In table 2, Glioma CSCs depend on OXPHOS for their energy production and

survival, and IMP2 (insulin-like growth factor 2 mRNA-binding protein 2) regulates OXPHOS in CSCs. The IMP2 is an effective target to develop anti-drug for CSCs. Depletion of IMP2 decreases their oxygen consumption rate that results in an impaired clonogenicity and tumorigenicity. Moreover, the combined use of mitochondria-targeted agents with conventional chemotherapeutics and other chemotherapeutic drugs, such as ROS scavenger inhibitors or ROS inducers, may be necessary to achieve maximum efficacy via CSCs elimination for cancer treatment.

What is the key to develop the mito-target drug which can affect to both common cancer cells and CSCs?

For now, it is unclear which target kills both common cancer cells and CSCs. However, by studying the mitochondrial features between CSCs and non-CSCs, we can identify targets to develop the effective anticancer drug for the elimination of CSCs, as well as common cancer cells. Moreover, the combined treatment of mitochondria-targeted agents with conventional chemotherapeutics can induce the cell death of common cancer cells and cancer stem cells.

(3) ***Reviewer 3 (01558248) recommended to make a table to show the differences of mitochondria.***

As recommended, we represented the differences of cancer stem cells mitochondria and non-cancer stem cells mitochondria in table 2.

Table 2. Mitochondrial features of cancer stem cells according to cancer origin

Cancer Origin	Mitochondria features			Energy metabolism of CSC	Target/drug for CSCs	Ref
	Feature	CSC	nonCSC			
Breast	Glucose uptake	High	Low	OXPHOS		[69]
	ATP contents	High	Low			
	OCR	High	Low			
	Lactate production	Low	High			
	Membrane potential	High	Low			
Glioma	Glucose consumption	Low	High	OXPHOS		[67]
	ATP contents	High	Low			
	Lactate production	Low	High			
	OCR	High	Low	OXPHOS	IMP-2	[68]
	ATP contents	High	Low			
Leukemia	ROS	Low	High	Low glycolysis	Bcl-2/	[64]

	Proliferation rate	Slow	Fast	Low OXPHOS	ABT263	
	OCR	Low	High			
	Lactate production	Low	High			
	ATP contents	Low	High			
Lung	Glucose consumption	Low	High			[63]
	OCR	Low	High			
	ROS level	Low	High			
	ATP contents	Low	High			
	Membrane potential	High	Low			
	Mitochondrial DNA	Low	High			
Ovarian					NV-128	[66]
	ROS	High	Low	OXPHOS		[65]
	Membrane potential	High	Low			
	ATP contents	High	Low			
	Glucose deprivation	Resist	Sensitive			

OCR; oxygen consumption rate, OXPHOS; oxidative phosphorylation, ABT263; Bcl-2 inhibitor, NV-128; isoflavone derivative (play a role as inhibitor of mitochondrial function), IMP-2; insulin-like growth factor 2 mRNA-binding protein 2.

(4) ***Reviewer 4 (01919991) suggest to cite and integrate in the review the following reference, Recent Pat Endocr Metab Immune Drug Discov, 2013; 7, 102-114.***

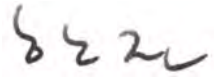
As suggested, we added a reference (Recent pat endocr Metab immune drug Discov, 2013;7, 102-114), in 21 line of page 12 ~ 1 line of page 13, as follows:

Meanwhile, it was reported that a drug which inhibits the self-renewal of CSCs by targeting of Notch and Hedgehog pathway has been developed [81]. It was also reported that has been developed a drugs, which can eliminate CSCs by targeting cell surface markers such as CD133 and EpCAM. However, the use of these drugs increases the exposure to side effects due to the sharing of signaling pathway and cell surface marker with normal stem cells. Thus, it is important to understand how CSCs differ from normal stem cells and differentiated cells. Moreover, a full understanding of the role of mitochondrial activity and energy metabolism in CSCs contributes to the development of the agents targeting mitochondrial functions (such as ROS overproduction, energy metabolism inhibition, and antioxidant protein inhibition), and presents a need to develop new strategies to target CSCs in the clinical field [81].

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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